Reference solution (b). Dissolve 20 mg of procaine hydrochloride R in reference solution (a) and dilute to 5 ml with reference solution (a).
Plate: TLC silica gel F$_{254}$ plate R.
Application: 5 µl.
Development: over a path of 10 cm.
Drying: in a current of warm air for 10 min.
Detection: spray with dimethylaminobenzaldehyde solution R7 and examine in ultraviolet light at 254 nm.
System suitability: reference solution (b):
- the chromatogram shows 2 clearly separated spots.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with reference solution (a).
D. Dilute 0.2 ml of solution S (see Tests) to 2 ml with water R.
The solution gives reaction (a) of chlorides (2.3.1).

TESTS
Solution S. Dissolve 5.0 g in carbon dioxide-free water R and dilute to 50 ml with the same solvent.
Appearance of solution. Solution S is clear (2.2.1) and not more intensely coloured than reference solution Y$_S$ (2.2.2, Method II).

pH (2.2.3); 4.5 to 6.0 for solution S.

Related substances. Liquid chromatography (2.2.29).
Buffer solution pH 2.5. Add 6 ml of perchloric acid solution R and 12 ml of dilute phosphoric acid R to 950 ml of water R. Adjust to pH 2.5 with a 40 g/l solution of sodium hydroxide R and dilute to 1000.0 ml with water R.
Test solution. Dissolve 10.0 mg of the substance to be examined in the mobile phase and dilute to 25.0 ml with the mobile phase.
Reference solution (a). Dilute 1.0 ml of the test solution to 20.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 100.0 ml with the mobile phase.
Reference solution (b). Mix 1.0 ml of the test solution with 1 ml of a 40 g/l solution of sodium hydroxide R and allow to stand for 20 min. Add 1 ml of dilute phosphoric acid R and dilute to 100.0 ml with the mobile phase. Dilute 25 ml of this solution to 100.0 ml with the mobile phase.

Column:
- size: l = 0.15 m, Ø = 3.9 mm;
- stationary phase: octadecylsilyl silica gel for chromatography R1 (5 µm) with a pore size of 10 nm and a carbon loading of 19 per cent;
- temperature: 35 °C.
Flow rate: 1 ml/min.
Detection: spectrophotometer at 309 nm.
Injection: 20 µl.
Run time: 4 times the retention time of oxybuprocaine.
Retention time: oxybuprocaine = about 9 min.
System suitability: reference solution (b):
- resolution: minimum 12 between the peaks due to oxybuprocaine and impurity B (hydrolysis product).

Limits:
- any impurity: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.1 per cent);
- total: not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.25 per cent);
- disregard limit: 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.0125 per cent).

Heavy metals (2.4.8): maximum 10 ppm.
12 ml of solution S complies with test A. Prepare the reference solution using lead standard solution (1 ppm Pb) R.

Loss on drying (2.2.32): maximum 0.5 per cent determined on 1.000 g by drying in an oven at 105 °C.

Sulphated ash (2.4.14): maximum 0.1 per cent, determined on 1.0 g.

ASSAY
Dissolve 0.300 g in a mixture of 20 ml of anhydrous acetic acid R and 20 ml of acetic anhydride R. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically (2.2.20).
1 ml of 0.1 M perchloric acid is equivalent to 34.49 mg of C$_7$H$_7$ClN$_2$O$_3$.

STORAGE
Protected from light.

IMPURITIES
A. R = H: 4-aminobenzoic acid,
B. R = O-CH$_2$-CH$_2$-CH$_2$: 4-amino-3-butoxybenzoic acid,
C. R = OH: 4-amino-3-hydroxybenzoic acid.

OXYBUTYNIN HYDROCHLORIDE
Oxybutynini hydrochloridum

C$_{17}$H$_{29}$ClN$_2$O$_3$ M, 394.0 [1508-65-2]

DEFINITION
4-(Diethylamino)but-2-ynyl (RS)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride.
Content: 99.0 per cent to 102.0 per cent (dried substance).

CHARACTERS
Appearance: white or almost white, crystalline powder.
Oxybutynin hydrochloride

**Solubility:** freely soluble in water and in ethanol (96 per cent), soluble in acetone, practically insoluble in cyclohexane.

**IDENTIFICATION**

First identification: B, D.

Second identification: A, C, D.

A. Melting point (2.2.14): 124 °C to 129 °C.

B. Infrared absorption spectrophotometry (2.2.24).

**Preparation:** discs.

**Comparison:** oxybutynin hydrochloride CRS.

C. Thin-layer chromatography (2.2.27).

**Test solution.** Dissolve 50 mg of the substance to be examined in ethanol (96 per cent) R and dilute to 10 ml with the same solvent.

**Reference solution (a).** Dissolve 10 mg of oxybutynin hydrochloride CRS in ethanol (96 per cent) R and dilute to 2 ml with the same solvent.

**Plate:** TLC silica gel plate R.

**Mobile phase:** methanol R.

**Application:** 5 µl.

**Development:** over a path of 15 cm.

**Drying:** in air.

**Detection:** expose to iodine vapour for 30 min.

**Results:** the principal spot in the chromatogram obtained with the test solution is similar in position, colour and size to the principal spot in the chromatogram obtained with the reference solution.

D. It gives reaction (a) of chlorides (2.3.1).

**TESTS**

**Solution S.** Dissolve 2.00 g in water R and dilute to 20.0 ml with the same solvent.

**Appearance of solution.** Solution S is clear (2.2.1) and not more intensely coloured than reference solution BY S (2.2.2, Method II).

**Optical rotation (2.2.7):** −0.10° to +0.10°, determined on solution S.

**Related substances.** Liquid chromatography (2.2.29).

**Test solution.** Dissolve 50.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 ml with the mobile phase.

**Reference solution (a).** Dissolve 5.0 mg of oxybutynin hydrochloride CRS and 5.0 mg of oxybutynin impurity A CRS in the mobile phase and dilute to 10.0 ml with the mobile phase. Dilute 5.0 ml of this solution to 50.0 ml with the mobile phase.

**Reference solution (b).** Dilute 1.0 ml of the test solution to 200.0 ml with the mobile phase.

**Column:**

- **size:** l = 0.15 m, Ø = 3.9 mm;
- **stationary phase:** octylsilyl silica gel for chromatography R2 (5 µm).

**Mobile phase:** mix 49 volumes of a solution containing 3.4 g/l of potassium dihydrogen phosphate R and 4.36 g/l of dipotassium hydrogen phosphate R and 51 volumes of acetonitrile R.

**Flow rate:** 1 ml/min.

**Detection:** spectrophotometer at 210 nm.

**Injection:** 10 µl.

**Run time:** twice the retention time of oxybutynin.

**Retention time:** oxybutynin = about 15 min; impurity A = about 24 min.

**System suitability:** reference solution (a):

- **resolution:** minimum 11.0 between the peaks due to oxybutynin and impurity A.

**Limits:**

- **impurity A:** not more than 1.5 times the area of the corresponding peak in the chromatogram obtained with reference solution (a) (1.5 per cent);
- **sum of impurities other than A:** not more than the area of the principal peak in the chromatogram obtained with reference solution (b) (0.5 per cent);
- **disregard limit:** 0.05 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.025 per cent).

**Heavy metals (2.4.8):** maximum 20 ppm.

12 ml of solution S complies with test A. Prepare the reference solution using 2 ml of lead standard solution (10 ppm Pb) R.

**Loss on drying (2.2.32):** maximum 3.0 per cent, determined on 1.000 g by drying in an oven at 105 °C.

**Sulphated ash (2.4.14):** maximum 0.1 per cent, determined on 1.0 g.

**ASSAY**

Dissolve 0.300 g in a mixture of 5.0 ml of 0.01 M hydrochloric acid and 50 ml of ethanol (96 per cent) R. Carry out a potentiometric titration (2.2.20), using 0.1 M sodium hydroxide. Read the volume added between the 2 points of inflexion.

1 ml of 0.1 M sodium hydroxide is equivalent to 39.4 mg of C22H32ClNO3.

**STORAGE**

Protected from light.

**IMPURITIES**

**Specified impurities:** A.

**Other detectable impurities** (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034)). It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use): B, C, D, E.

**A.** 4-(diethylamino)but-2-ynyl (RS)-2-(cyclohex-3-ynyl)-2-cyclohexyl-2-hydroxyacetate,

**B.** 4-(diethylamino)but-2-ynyl 2-hydroxy-2,2-diphenylacetate (diphenyl analogue of oxybutynin),
A. Infrared absorption spectrophotometry (2.2.24).
Preparation: discs.
Dissolve 50 mg in water R and dilute to 5 ml with the same solvent. Render the solution alkaline with dilute ammonia R1. Allow the mixture to stand until a precipitate is formed. Filter, wash the precipitate with 10 ml of cold water R, and dry for 1 h at 105 °C. Examine the precipitate.
Comparison: repeat the operations using 50 mg of oxycodone hydrochloride CRS.

B. It gives reaction (a) of chlorides (2.3.1).

TESTS
Solution S. Dissolve 1.00 g in carbon dioxide-free water R and dilute to 50.0 ml with the same solvent.
Acidity or alkalinity. To 10 ml of solution S add 0.05 ml of methyl red solution R. Not more than 0.2 ml of 0.02 M sodium hydroxide or 0.02 M hydrochloric acid is required to change the colour of the indicator.
Specific optical rotation (2.2.7): −140 to −148 (anhydrous substance), determined on solution S.
Related substances. Liquid chromatography (2.2.29).
Prepare the solutions protected from light.
Test solution. Dissolve 0.100 g of the substance to be examined in a 1 per cent V/V solution of dilute acetic acid R and dilute to 50.0 ml with the same solvent.
Reference solution (a). Dissolve 20.0 mg of oxycodone hydrochloride CRS in a 1 per cent V/V solution of dilute acetic acid R and dilute to 10.0 ml with the same solution.
Reference solution (b). To 1.0 ml of the test solution, add 1.0 ml of reference solution (a) and dilute to 100.0 ml with a 1 per cent V/V solution of dilute acetic acid R. Dilute 1.0 ml of the solution to 10.0 ml with a 1 per cent V/V solution of dilute acetic acid R.

Column:
— size: l = 0.15 m, Ø = 4.6 mm,
— stationary phase: octadecylsilica gel for chromatography R (5 µm),
— temperature: 40 °C.
Mobile phase:
— mobile phase A: mix 830 ml of a 1.1 g/l solution of sodium heptanesulphonate monohydrate R previously adjusted to pH 2.0 with a mixture of equal volumes of phosphoric acid R and water R, with 70 ml of acetonitrile R and 100 ml of methanol R;
— mobile phase B: mix 600 ml of a 1.1 g/l solution of sodium heptanesulphonate monohydrate R previously adjusted to pH 2.0 with a mixture of equal volumes of phosphoric acid R and water R, with 150 ml of acetonitrile R and 250 ml of methanol R;

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mobile phase A (per cent V/V)</th>
<th>Mobile phase B (per cent V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 → 60</td>
<td>100 → 50</td>
<td>0 → 50</td>
</tr>
<tr>
<td>60 → 62</td>
<td>50 → 100</td>
<td>50 → 0</td>
</tr>
<tr>
<td>62 → 70</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Flow rate: 1.5 ml/min.
Detection: spectrophotometer at 230 nm.
Injection: 20 µl.
Relative retention with reference to oxycodone (retention time = about 24 min): impurity A = about 0.4; impurity B = about 0.7; impurity C = about 1.14; impurity D = about 1.18; impurity E = about 1.18; impurity F = about 2.4.
System suitability: reference solution (b):
— resolution: minimum 3 between the peaks due to oxycodone and impurity D.
Limits:
— correction factor: for the calculation of content, multiply the peak area of impurity F by 0.5;
— sum of impurities D and E: not more than 10 times the area of the peak due to oxycodone in the chromatogram obtained with reference solution (b) (1.0 per cent);